



# First report of *Coenosia attenuata* (Stein, 1903) (Diptera, Muscidae) in Uruguay, confirmed by DNA barcode sequences

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## Abstract

*Coenosia attenuata* (Stein, 1903) is a predatory fly which feeds on other insects and can be used as a possible biological control agent. We report this insect in Uruguay for the first time. The flies were found in greenhouses on tomatoes and sweet peppers and identified using both DNA barcoding and morphological characteristics.

## Keywords

Biological control, Coenosiinae, COI, horticulture, predator

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## Introduction

The predatory fly *Coenosia attenuata* (Stein, 1903) (Muscidae, Coenosiinae) is a polyphagous predator, and some of its prey are common pests of greenhouse and outdoor crops. The larvae live in the soil and feed on small soft-bodied invertebrates, such as larvae of Sciariidae and Ephydriidae (Diptera) (Mateus 2012). Adults live on the ground and feed on flying insects which generally cause damage to crops, such as *Bemisia tabaci* (Gennadius, 1889), *Trialeurodes vaporariorum* Westwood, 1856, *Bactericera cockerelli* (Šulc, 1909), and *Tuta absoluta* (Meyrick, 1917), among others (Mateus 2012; Martins et al. 2012; Bautista-Martínez et al. 2017). Adults wait for their prey to land on plants or other surfaces or attack prey which fly nearby in an ambush hunting behavior. An interesting behavior of this predator is that it attacks and kills prey even when it is not hungry (Martínez and Cocquempot 2000). Its ability to tolerate

high temperatures while maintaining predatory activity makes it especially interesting for biological control of pests in greenhouses and in warm environments (Gilioli et al. 2005).

*Coenosia attenuata* is native from southern Europe (Hennig 1964). It is currently distributed in several countries of Europe, Asia, Africa, and Oceania (Pohl et al. 2012). In the Americas, this fly was recorded for the first time in Ecuador, Peru, and the United States in 2002 (Martínez-Sánchez et al. 2002; Hoebke et al. 2003). Later, it was found in Canada (Sensenbach 2004), Colombia (Pérez 2006), Costa Rica (Hernández 2008), Chile (Couri and Salas 2010), Mexico (Bautista-Martínez et al. 2017), Venezuela (Solano et al. 2017), Brazil (Couri et al. 2018), and Honduras (Orozco 2018).

In this work, we report the presence of *Coenosia attenuata* (Stein, 1903) in Uruguay for the first time

by using DNA barcoding and taxonomic identification methods.

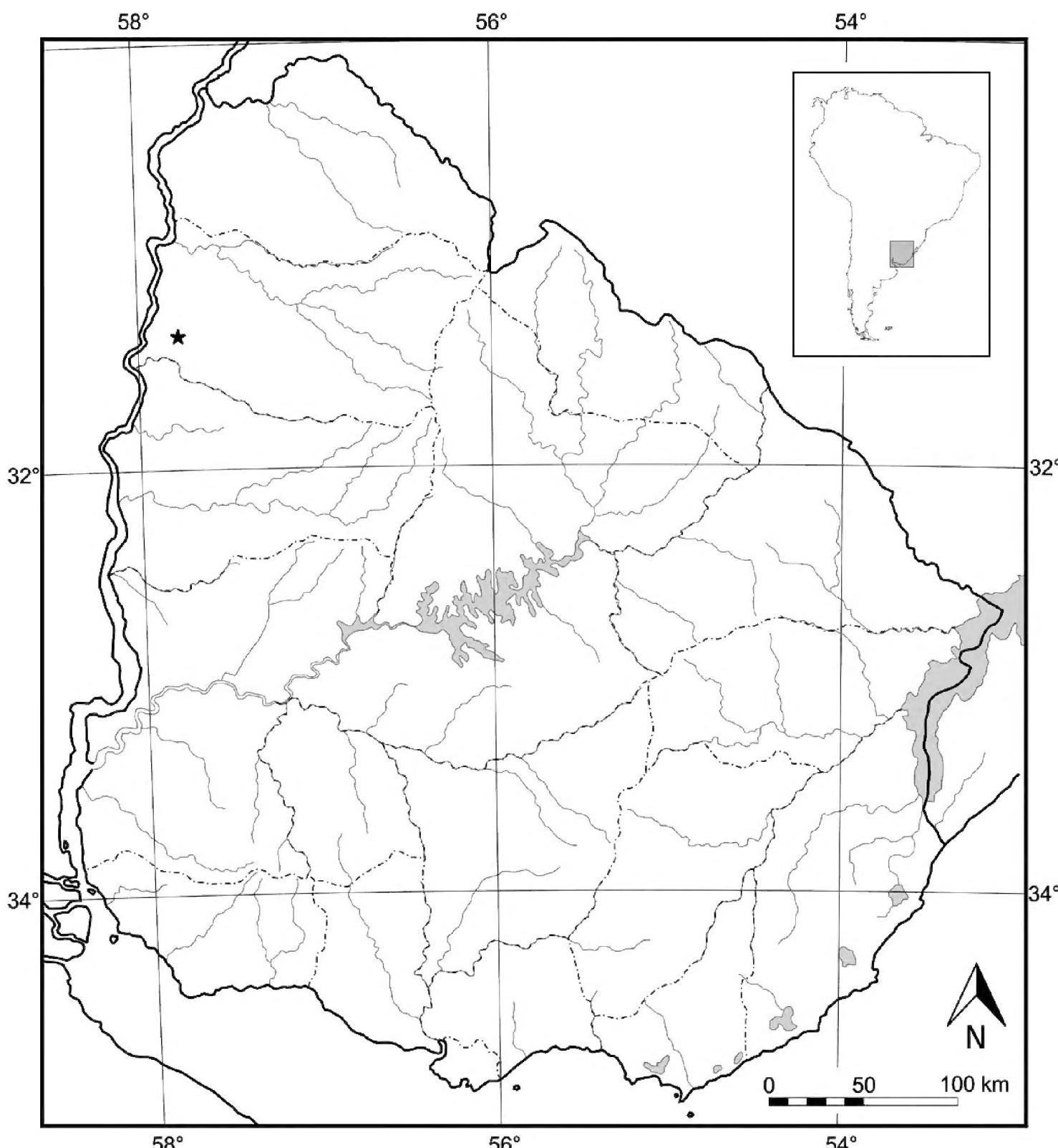
## Methods

During 2019, we collected specimens of adult flies from tomato and sweet pepper crops from greenhouses in Salto, Uruguay (Fig. 1). The geographical coordinates were obtained using Google Earth® v. 7.3.2 (geodetic datum: WGS84). Morphological identification of the specimens was based on Pohl et al. (2012). The samples were deposited in the Instituto Nacional de Investigación Agropecuaria (INIA), Salto Grande's Insects Collection. The insects used for the DNA barcode were photographed laterally, dorsally, and ventrally and labeled appropriately before DNA extraction.

Total DNA was extracted from individual samples according to a standard CTAB method described by Doyle and Doyle (1987). In this case, after being photographed, the entire body of the fly was used for DNA extraction. The mitochondrial cytochrome *c* oxidase subunit I gene (COI) was amplified and sequenced using primers

LepFoIF RKTCAACMAATCATAAAGATATTGG and COISG-R TAAACTTCTGGRTGWCCAAAAATCA. The PCR mix contained approximately 200 ng of total cell DNA, 10 pmol of each primer, 200 µmol of each dNTP, 2.5 µl of PCR buffer (with 20 mmol MgCl<sub>2</sub>), 0.8 units Taq DNA polymerase (Thermo Fisher Scientific, USA), ultrapure water was added for a final volume of 25 µl. Temperature cycling was as follows: 95 °C (4 min), 40 cycles of 95 °C (50 sec), 52 °C (50 sec), 72 °C (50 sec), and a final extension at 72 °C (5 min).

PCR products were purified by the standard method described by Sambrook and Russell (2006) and sent to Macrogen Inc. (Seoul, Korea) sequencing service (<http://www.macrogen.com/>). The resulting sequences were analyzed using a sequence alignment editor (BioEdit v. 7.2.5; Hall 1999). The taxonomic affinities of our nucleotide sequences were identified using the Basic Local Alignment Search Tool, BLAST (Johnson et al. 2008) and the Barcode of Life Data Systems (Ratnasingham and Hebert 2007). The obtained sequences have been deposited in GenBank and their accession numbers are MN880872 and MN909948.



**Figure 1.** Map of Uruguay and the geographical location of the collection site (star icon) for the first record of *Coenosia attenuata* (Stein, 1903).

## Results

### *Coenosia attenuata* (Stein, 1903)

**New records.** URUGUAY • 1♀; Salto; 31°21'07.3"S, 057°51'03.4"W; 16 Oct. 2019; Rodríguez leg.; greenhouse with tomato; COI; GenBank MN880872; specimen code MG2\_coiSG • 1♂; same collection data as for preceding; 31°21'07.8"S, 057°51'00.8"W; greenhouse with sweet pepper; GenBank MN909948; specimen code M4\_coiSG.

**Identification.** The flies are identified as *C. attenuata* (Stein, 1903). All morphological characteristics match the redescription of this species by Pohl et al. (2012). The flies measure approximately 2.5–5.00 mm in length. The female, which is slightly larger and darker than the male, has black antennae, its femora is largely black, and its abdomen is distinct with black bands. The male has yellow antennae. Legs, face, and frons are silvery white and abdomen has slightly pronounced black bands (Fig. 2).

The samples MG2\_coiSG, from tomato crop, and M4\_coiSG, from sweet pepper crop, were successfully sequenced for the DNA barcode region of COI, with a sequence length of 658 bp (0% ambiguities) with no evidence of stop codons or contamination. Using the BOLD ID Engine, the comparison with the DNA barcode library resulted in a 100% pairwise nucleotide match over 90

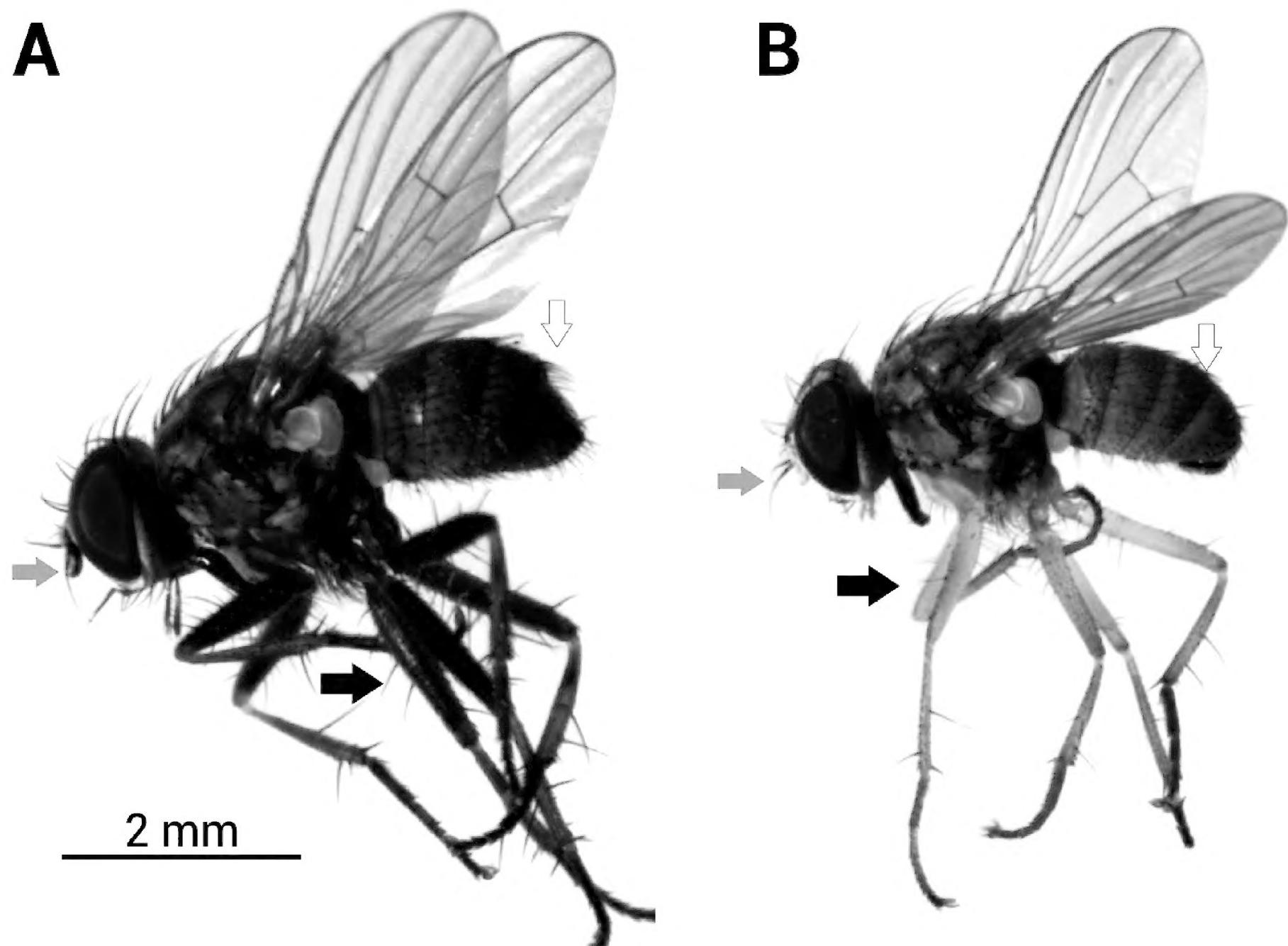
other *C. attenuata* specimens housed in BOLD, confirming their primary identification. NCBI BLAST analysis retrieved 76 *C. attenuata* nucleotide sequences showing 100% nucleotide identity to our sequences MN880872 and MN909948.

## Discussion

The presence of *Coenosia attenuata* on most continents, its ability to live in high-temperature environments, and its predatory behavior make it an excellent biological control agent (Martínez and Cocquempot 2000; Pohl et al. 2012). In addition, this species adaptable to laboratory conditions and can be bred in captivity for release in commercial crops (Martins et al. 2015).

We have observed *C. attenuata* hunting *Bemisia tabaci* and *Trialeurodes vaporariorum* in sweet pepper and tomato crops. We saw it using the ambush technique to catch its prey in mid-flight, as described by Martínez and Cocquempot (2000). This is an interesting observation, as *Bemisia tabaci*, *Trialeurodes vaporariorum*, and other reported prey are important pests that affect greenhouse and field crops in Uruguay.

As the identification of *C. attenuata* and its prey in the field is difficult, DNA barcoding is a valuable tool for determining predator and prey. This technique allows for



**Figure 2.** Lateral photograph of *Coenosia attenuata* (Stein, 1903) collected from Uruguay. **A.** Female, MG2\_coiSG. **B.** Male, M4\_coiSG. Arrows indicate morphological differences between female and male: femur (black), antennae (grey), abdomen color (white). Female individuals are larger.

*C. attenuata* and other species of insects to be identified with great precision (Orozco 2018). This is highly valuable if included as a routine process when documenting the presence of taxa across their geographical range. It enables rapid and automated species identification of adult or immature individuals with the same precision (Hebert et al 2003).

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## Authors' Contributions

This paper was originally conceived by MG and JB. The study was designed by MG, AA, and JB. MG and AR carried out the fieldwork and processed collected material; MG and AA identified the specimens by barcoding. All authors wrote the final version of the manuscript.

## References

- Bautista-Martínez N, Illescas C, García C (2017) First report of “hunter fly” *Coenosia attenuata* (Diptera: Muscidae) in Mexico. Florida Entomologist 100: 174–175. <https://doi.org/10.1653/024.100.0126>
- Couri M, Salas C (2010) First record of *Coenosia attenuata* Stein (Diptera: Muscidae) from Chile with biological notes. Revista Brasileira de Entomologia 4: 144–145. <https://doi.org/10.1590/S0085-56262010000100020>
- Couri M, Sousa V, Lima R, Dias N (2018) The predator *Coenosia attenuata* Stein (Diptera, Muscidae) on cultivated plants from Brazil. Anais da Academia Brasileira de Ciências 90: 179–183.
- Doyle J, Doyle J (1987) A rapid DNA isolation procedure for small quantities offresh leaf tissue. Phytochemical Bulletin 19: 11–15.
- Gilioli G, Baumgartner J, Vacante V (2005) Temperature influences on functional response of *Coenosia attenuata* (Diptera: Muscidae) individuals. Journal of Economic Entomology 98: 1524–30. <https://doi.org/10.1603/0022-0493-98.5.1524>
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.
- Hennig W (1964) Muscidae. In: Lindner E (Ed.) Die Fliegen der Paläarktischen Region Volume 7. Schweizerbart Science Publishers, Stuttgart, 1955–1964.
- Hebert P, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proceedings of the Royal Society B, Biological Sciences 270: 313–321. <https://doi.org/10.1098/rspb.2002.2218>
- Hernández J (2008) Presencia de la mosca tigre en Costa Rica. Actuabilidad Fitosanitaria 33: 3.
- Hoebeke E, Sensenbach E, Sanderson J, Wright S (2003) First report of *Coenosia attenuata* Stein (Diptera: Muscidae), an old world “hunter fly” in North America. Proceedings of the Entomological Society of Washington 105: 769–775.
- Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S, Madden TL (2008) NCBI BLAST: a better web interface. Nucleic Acids Research 36: W5–W9. <https://doi.org/10.1093/nar/gkn201>
- Martinez M, Cocquempot C (2000) La mouche *Coenosia attenuata*, nouvel auxiliaire prometteur en culture protégée. Revue Horticole 414: 50–52.
- Martínez-Sánchez A, Marcos García M, Pont A (2002) *Coenosia attenuata* Stein 1903 (Diptera: Muscidae) nueva especie para la fauna neotropical. Bollettino di Zoología Agraria ed Bachicoltura 34: 269–272.
- Martins J, Domingos C, Nunes R, García A, Ramos C, Mateus C, Figueiredo E (2012) *Coenosia attenuata* (Diptera: Muscidae), um predador em estudo para utilização em culturas protegidas. Revista de Ciências Agrárias 35: 229–235.
- Martins J, Mateus C, Ramos AC, Figueiredo E (2015) An optimized method for mass rearing the tiger-fly, *Coenosia attenuata* (Diptera: Muscidae). European Journal of Entomology 112: 1–7. <https://doi.org/10.14411/eje.2015.059>
- Mateus C (2012) Bioecology and behaviour of *Coenosia attenuata* in greenhouse vegetable crops in the Oeste region, Portugal. Bulletin of Insectology 65: 257–263.
- Orozco J (2018) Código de barras de ADN revela el primer registro de *Coenosia attenuata* Stein (Diptera: Muscidae) en Honduras. Ceiba 55: 70–72. <https://doi.org/10.5377/ceiba.v55i1.5456>
- Pérez M (2006) Estudio de la morfología externa de los adultos de la mosca cazadora *Coenosia attenuata* Stein, 1903 (Diptera: Muscidae) y primer reporte para Colombia. Revista de la Facultad de Ciencias Básicas 2: 67–87.
- Pohl D, Kühne S, Karaca İ, Moll E (2012) Review of *Coenosia attenuata* Stein and its first record as a predator of important greenhouse pests in Turkey. Phytoparasitica 40: 63–68.
- Ratnasingham S, Hebert P (2007) BOLD: the Barcode of Life Data System (<http://www.barcodinglife.org>). Molecular Ecology Notes 7: 355–364. <https://doi.org/10.1111/j.1471-8286.2007.01678.x>
- Roy M, Fréchette M (2006) Première mention de *Coenosia attenuata* (Diptera: Muscidae) dans les serres au Québec. Laboratoire de Diagnostic en Phytoprotection. Ministère de l’Agriculture, des Pêcheries et de l’Alimentation, Quebec, Canada.
- Sambrook J, Russell D (2006) The condensed protocols: from molecular cloning: a laboratory manual. Cold Spring Harbor, New York, 455–502 pp.
- Sensenbach E (2004) *Coenosia attenuata* Stein (Diptera: Muscidae): a predatory fly in North American greenhouses. Master’s thesis, Cornell University, Ithaca, New York, 83 pp.
- Solano Y, Pont A, De Freitas J, Moros G, Goyo Y (2017) First record of *Coenosia attenuata* Stein, 1903 (Diptera: Muscidae) in Venezuela. Anales de Biología 39: 223–226. <https://doi.org/10.6018/analesbio.39.23>